

REMARKS

Claims 7-10 and 12 - 24 are pending.

No new matter is added by this amendment.

I.     Claim Objections

New claims 18, 23 and 24 have been objected to because they do not consistently use recombinant adeno-associated virus or rAAV.

The claims have been amended to correct this inconsistency. This amendment does not narrow the scope of the claims.

Reconsideration and withdrawal of this objection is requested.

II.    Rejections Under 35 USC §112

Claims 7-10 and 12-24 have been rejected under 35 USC §112, first and second paragraphs. The examiner has based these rejections on the language in claims 7, 12 and 13 and new claims 18, 23 and 24 which recite "wherein the recombinant AAV is at least as free of the contaminating adenoviral helper virus as is obtained by subjecting said recombinant AAV to four rounds of cesium chloride gradient centrifugation". The examiner reiterates that the specification does not provide written support in the specification as what would be the contaminating levels of adenoviral helper virus after four rounds of cesium chloride centrifugation.

Applicants respectfully traverse this rejection.

The specification teaches that, as measured by the assay described on page 35, lines 1-5, rAAV purified according to the invention, contains *no* detectable amounts of contaminating adenovirus. Given this description, Applicants submit that the meaning of this language is clear and definite.

The specification describes a method of detecting the amount of contaminating adenovirus in a rAAV preparation. See, page 35, lines 1-5, which summarize a method described in Fisher et al, J. Virol, 70:520-532 (1996), which is incorporated by reference in the present application and also in the priority documents. In addition, various other methods for detecting contaminating adenovirus, e.g., PCR analysis, have been known to those of skill in the art as of the priority date of this application.

Thus, the specification provides sufficient teachings as to permit one of skill in the art to determine the amount of contaminating adenovirus in a rAAV preparation, regardless of the method by which the rAAV preparation is purified.

The inventors are the first to have found that it is the contamination of rAAV preparations with adenoviral helper virus which induces an immune response upon delivery of rAAV delivery vectors which are so contaminated. Thus, the present inventors are the first to have described that it is the purification of rAAV away from adenoviral helper-virus that causes reduction or elimination of a cytotoxic immune response following rAAV delivery. See, e.g., Figs 5A-C, Example 5 and Example 7. Applicants have shown that four rounds of cesium chloride gradient centrifugation is a method for providing an rAAV preparation with a level of purity from adenoviral helper virus which eliminates cytotoxic immune response. However, as discussed above, four rounds of cesium chloride gradient centrifugation is not the only method by which adequate levels of contaminating adenoviral helper can be removed and the invention should not be limited to purification by only this number of steps of this particular purification method.

More particularly, given the teachings in the specification, one of skill in the art can readily determine both quantitatively and/or qualitatively the meaning of the phrase "at least as free of the contaminating adenoviral helper virus" as used in the present invention to describe the level of purity of the recombinant AAV of the invention.

Reconsideration and withdrawal of these rejections is requested.

### III. Double Patenting

Claims 7-10 and 12-24 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-4 of US Patent No. 5,866,552; provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-6, 9, 20, 21, 23, 25, 26 and 27 of co-pending Application No. 09/237,064 and claims 18-24, 26-28, and 30-35 of co-pending Application No. 09/242,977.

Applicants agree to file a terminal disclaimer over the '552 patent. Applicants also agree to file terminal disclaimers with respect to each of the co-pending applications contingent upon the provisional nature of the rejection being removed prior to issuance of this application.

IV. Rejection under 35 USC §102(e).

Claims 7-10, 18 and 23 are rejected under 35 USC §102(e) as being anticipated by Podskoff et al, US Patent 5,858,351.

Applicants respectfully traverse this rejection.

Podskoff fails to recognize that contamination of rAAV with adenoviral helper viruses is responsible for an undesired cytotoxic immune response. It is applicants who were the first to discover the cause of this problem. Podskoff fails to recognize that his rAAV preparations are insufficiently purified to avoid this undesired immune response.

Podskoff describes the use of only one round of cesium chloride gradient centrifugation, followed by heat inactivation of residual helper virus [col. 14, line 65-col. 15, line 3 and col. 18, 26-35 of '351 patent]. Thus, Podskoff recognizes that there is detectable adenovirus helper remaining in the cesium chloride banded preparation.

With regard to the present invention, it is noted in Fisher et al, J. Virol., 70(10):520-532 (1996), which is incorporated by reference for the purification method described therein [see, specification, page 15, line 12; page 34, lines 6 and 21-21], that:

"Routinely, the purification scheme described above removed all detectable H5.CBALP [adenoviral] helper virus by the third round of buoyant density ultracentrifugation." Page 521, column 2, last sentence of paragraph beginning in column 1.

This teaching makes it clear that at least three rounds of CsCl centrifugation are required to remove helper virus from rAAV preparations. The present application

describes the performance of yet an additional (i.e., fourth) round of CsCl centrifugation to ensure that any possible remaining contaminants have been removed.

Further evidence that the compositions of the present invention differ in purity from that of Podskoff is found in the amount of wild-type AAV found in the preparations. The present invention provides compositions which are 2 logs more pure of contaminating wild-type (wt) AAV than Podskoff. Note, when purified as described in the present invention, rAAV preparations contain <1 infectious unit wt AAV per  $10^9$  genomes rAAV. See, page 35, lines 5-6 of the specification. In contrast, Podskoff detects wt AAV contamination of approximately 1 in  $10^7$ . See, col. 19, lines 16-17 of the '351 patent.

It is only the inventors who have recognized the significance of eliminating adenoviral contamination *and not just contamination by adenoviral function* which led to the present invention.

For these reasons, applicants request withdrawal of the rejection.

V. Rejections Under 35 USC §103

- A. Claims 7-10 and 18-24 have been rejected under 35 USC §103(a) as being unpatentable over Podskoff, in view of Kashyap, J. Clin. Invest., 96:1612-1620 (1995).

Podskoff teaches only the use of a rAAV vector. Kashyap contains no teaching regarding the use of such a rAAV vector. The combined teachings of Podskoff and Kashyap fail to recognize the level of purity necessary for an rAAV to obtain the results provided by the present invention. Thus, even if the teachings of these references are combined as suggested by the examiner, the present invention is not obvious.

Fisher et al, makes it clear that at least three rounds of CsCl centrifugation are required to remove helper virus from rAAV preparations. The present application describes the performance of yet an additional (i.e., fourth) round of CsCl centrifugation to ensure that any possible remaining contaminants have been removed.

Further, the present invention provides compositions which are 2 logs more pure of contaminating wild-type (wt) AAV than Podsakoff. Note, when purified as described in the present invention, rAAV preparations contain <1 infectious unit wt AAV per  $10^9$  genomes rAAV. See, page 35, lines 5-6 of the specification. In contrast, Podsakoff detects wt AAV contamination of approximately 1 in  $10^7$ . See, col. 19, lines 16-17 of the '351 patent. One of skill in the art will readily understand the removal of wt AAV reduces (or eliminates) the possibility of homologous recombination between the wtAAV and the rAAV of the invention.

Thus, the rAAV of the present invention are more pure than taught by Podsakoff. Again, it is noted that Kashyap fails to suggest anything about the use of rAAV vectors. Thus, the combination is deficient.

In addition, neither of the cited documents recognizes the importance of removing contaminating adenoviral helper from rAAV preparation to the level of purity required by the present invention. Notably, following Podsakoff's rAAV purification, he teaches heat-inactivation which will destroy the function of Ad. Thus, Podsakoff appears to be focused upon eliminating the function of the contaminating adenoviral helper, rather than removing the adenoviral helper itself. See, Podsakoff, col. 1, lines 52-55: “[A]denovirus vectors express viral proteins that may elicit an immune response which may decrease the life of the transduced cell.”. Further, Podsakoff postulates that “the adult muscle cell may lack the receptor which recognizes adenovirus vectors, precluding efficient transduction of this cell type using such vectors.” [Col. 1, lines 58-56]. Thus, Podsakoff’s motivation is to use rAAV because he believes that there are insufficient adenoviral receptors in muscle cells. Podsakoff does not recognize that the mere presence of contaminating adenoviruses (even in the absence of the ability to express adenoviral proteins) may cause an immune response. Thus, Podsakoff does not suggest a solution which avoids even heat-inactivated adenoviral contaminants.

It is only the inventors who have recognized the significance of eliminating adenoviral contamination *and not just contamination by adenoviral function* which led to the present invention.

The combined teachings of the cited documents do not suggest the present invention.

For these reasons, even if combined, Podsakoff and Kashyap fail to suggest the present invention.

Reconsideration of this rejection is requested.

- B. Claims 7-20 and 18-24 have been rejected under 35 USC §103(a) as being unpatentable over Podsakoff, in view of Fang, Hu Gene Therapy, 6:1039-1044 and Kay et al, US Patent 5,980,886.

As discussed above, Podsakoff fails to recognize that the contamination of rAAV preparations with helper virus causes an undesired cytotoxic immune response. Further, Podsakoff fails to teach or suggest any method of achieving a sufficient level of purification of rAAV from helper adenovirus to provide an “rAAV which is at least as free of contamination with helper adenovirus as is achieved by four rounds of cesium chloride gradient centrifugation”. Neither Fang nor Kay teach or suggest delivery of rAAV vectors. Thus, secondary references can not be combined with the teachings of Podsakoff in any manner which would overcome the deficiencies of the Podsakoff.

The combined teachings of Podsakoff, Fang and Kay fail to recognize that rAAV preparations contaminated with adenoviral helper virus causes an immune response to be induced. The combined teachings of the cited documents also fails to suggest any method by which this defect in the prior art rAAV preparations could be corrected. The problem is first recognized by applications. The solution provided by the present invention is nonobvious.

Reconsideration and withdrawal of the rejection is requested.

The Director of the U. S. Patent and Trademark Office is hereby authorized to charge any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees to Deposit Account No. 08-3040.

Respectfully submitted,

HOWSON AND HOWSON  
Attorneys for the Applicants

By Cathy A. Kodroff  
Cathy A. Kodroff  
Registration No. 33,980  
Spring House Corporate Center  
Box 457  
Spring House, PA 19477  
Telephone: (215) 540-9210  
Telefacsimile: (215) 540-5818

**Appendix A**  
**“Marked-up Version of Amended Claims”**

12 (Twice Amended). A recombinant adeno-associated virus (rAAV) comprising sequences encoding factor IX and regulatory control sequences which permit expression of factor IX in a cell, wherein the rAAV is at least as free of adenoviral helper virus as is obtained by subjecting said recombinant AAV to four rounds of cesium chloride gradient centrifugation.

18 (Amended). A method of delivering a transgene to a mammal comprising the step of:

administering intramuscularly to a mammal a composition comprising a biologically compatible carrier and a recombinant adeno-associated virus (rAAV) comprising a transgene encoding a secretable protein operably linked to sequences which control expression thereof, [wherein said rAAV is substantially free of contamination with an adenovirus,] wherein said rAAV is at least as free of adenoviral helper virus as is obtained by subjecting said [recombinant] rAAV to four rounds of cesium chloride gradient centrifugation, whereby the protein is secreted from rAAV-transduced muscle cells.

23(Amended). The method according to claim 18, wherein the level of contaminating adenoviral helper virus is the same as that obtained by subjecting said [recombinant] rAAV to four rounds of cesium chloride centrifugation.

24(Amended). The composition according to claim 13, wherein the level of contaminating adenoviral helper virus is the same as that obtained by subjecting said [recombinant] rAAV to four rounds of cesium chloride centrifugation.